NOTICE

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Purification and Cloning of a Soluble ATP-Diphosphohydrolase (Apyrase) from Potato Tubers (*Solanum tuberosum*)[1]

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A soluble ATP-diphosphohydrolase (apyrase, EC 3.6.1.5) has been purified from potato tubers, *Solanum tuberosum*, to a specific activity of 10,000 μmol P_i/mg/min. The cDNA corresponding to the potato apyrase has been isolated and termed RROP1. The deduced amino acid sequence contains a putative signal sequence, two hydrophobic regions at the carboxy terminus, two potential Asn-linked glycosylation sites, and four regions in the amino-terminal half that we term ACR (apyrase conserved regions) 1–4 that are highly conserved in known apyrases and related enzymes: garden pea nucleoside triphosphatase, *Toxoplasma gondii* nucleoside triphosphate hydrolases, and *Saccharomyces cerevisiae* golgi guanosine diphosphatase. A yeast 71.9-kDa hypothetical protein on chromosome V, a *Caenorhabditis elegans* hypothetical 61.3-kDa protein on chromosome III, and human CD39, a lymphoid cell activation antigen, also share the conserved ACR regions, but their ability to hydrolyze nucleotides has not been assessed.

ATP-diphosphohydrolase (apyrase) catalyzes the hydrolysis of phosphoanhydride bonds of nucleoside tri- and di- phosphates in the presence of divalent cations. This enzyme is distinct from other phosphohydrolases in that it has a high specific activity, broad nucleotide substrate specificity, broad divalent cation requirement, and insensitivity to known inhibitors of various ATPases (P-type, F-type, and V-type), phosphatases, and adenylate kinase. The family of proteins that share these characteristics has been termed E-type ATPases (1). Apyrase activities are found in a wide variety of organisms, tissue types, and cell types, as reviewed by Plesner (1). In animals, the enzyme exists as a plasma membrane ecto-ATPase in cell types such as rat hepatocytes (2), rabbit skeletal muscle transverse tubules (3), and chicken gizzard smooth muscle (4). Recently, soluble apyrases from the dense granules of a protozoan parasite, *Toxoplasma gondii* (5,6), and from mosquito saliva (*Aedes aegypti*) (7) have been cloned.

The physiological function(s) of the apyrases are not known, but various investigators have put forth hypotheses based on the extracellular nature of the enzymatic activity and the physiology of extracellular nucleotides. Possible functions include: 1) the concomitant regulation of P_2-purinergic receptors that recognize ATP and P_1-purinergic receptors that recognize adenosine; 2) the salvaging of extracellular adenosine through the sequential hydrolysis of ATP to AMP by apyrase, AMP to adenosine by 5′-nucleotidase, and subsequent import through nucleoside transporters; 3) the regulation of ecto-kinase substrate concentration, and 4) the inhibition of ADP-induced platelet aggregation (1,2,8–12).

Potato apyrase was first characterized by Kalckar (13) as an adenylpyrophosphatase and was later termed apyrase by Meyerhof (14). A number of isoapyrases with varying kinetic and subcellular localization properties have been identified from different varieties of *Solanum tuberosum* (15–19). Potato apyrase has been speculated to be a regulator of various steps involved in starch synthesis, as glycosyltransferases, ADP-glucose pyrophosphorylase, and other starch metabolism enzymes are regulated by the levels of either ATP, ADP, or P_i (20–22).

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We have cloned the cDNA for the potato apyrase to further investigate the structure/function relationship of the potato apyrase polypeptide. Sequence comparisons with other apyrases and related proteins show the existence of several conserved regions, suggesting the apyrases are an evolutionarily related family of proteins.

MATERIALS AND METHODS

Apyrase purification. The soluble apyrase was isolated from red skin potatoes (*Solanum tuberosum*) obtained from a local food market (Star Market, MA). The enzyme was purified according to Kettlun *et al.* (21) with an additional sucrose density centrifugation step. The sucrose gradient consisted of a 10%–30% continuous gradient in 50 mM potassium succinate, 400 mM potassium chloride, 1 mM thioglycolic acid, pH 4.0. The gradient was centrifuged at 180,000 × g in an SW 50.1 rotor for 24 hours.

Apyrase activity. Apyrase activity was determined by the release of orthophosphate from ATP as described by Fiske and Subbarow (23). The enzymatic activity is expressed as μmol P_i/mg of protein/minute. Approximately 10 ng – 20 μg of total protein, quantitated using the method described by Bradford (24), from the various purification steps were used in the assay.

Microsequencing. The purified apyrase was analyzed for amino terminal and trypsin digested peptide sequences by William S. Lane (Harvard Microsequencing Facility, Harvard University, Cambridge, MA). The tryptic digested peptides were separated on an HPLC column and five peptides were sequenced.

PCR amplification. Degenerate oligonucleotides were designed from the amino and carboxy terminal ends in the sense and antisense orientations, respectively, of the two longest peptides (Fig. 2, peptides #1 and #2).

PCR reactions were performed as described by Maniatis *et al.* (25) at an annealing temperature of 50°C using single stranded potato tuber cDNA as template. Total RNA was isolated from potato tubers (var. Red dale, Johnny's, Maine) using the method of Logemann *et al.* (26). Poly-A RNA was further purified using the PolyATract mRNA Systems II Kit (Promega). Single stranded cDNA was synthesized from ~10 μg poly-A RNA using Superscript Reverse Transcriptase (Gibco BRL).

cDNA sequences corresponding to both peptides were amplified, cloned into the polylinker site of pGEM 3Zf(−), and sequenced from the T7 and Sp6 promoters using the Sequenase Version 2.0 Kit (U.S. Biochemical).

Non-degenerate sense and antisense primers from peptides #1 and #2, respectively, produced a PCR amplified product of 131 bp. This DNA was sequenced as above. All oligonucleotides were made by Michael Berne (Tufts Microchemistry Facility, Tufts University Medical School, Boston, MA).

cDNA library screening. Potato tuber cDNA libraries in λ_{ZAP} were generously provided by Dr. Thomas Okita (Washington State University, WA) and Dr. Normand Brisson (University of Montreal, Quebec). 10,000 pfu were screened from the Brisson library. The 131 bp DNA was random primed using the NEBlot Kit (NEB) and [α-^{32}P]dATP (NEN, 3000 Ci/mmole) to a specific activity of ~10^9 dpm/μg. Hybridization and washing were done as described by Maniatis *et al.* (25). A single insert was isolated, and the Bluescript plasmid was excised using the ExAssist/SOLR *in vivo* excision protocol (Stratagene). The cDNA was sequenced on both strands with regularly spaced internal sense and antisense primers.

Nucleic acid and amino acid sequence alignments. The apyrase DNA sequence and the deduced amino acid sequence were checked for homology to known sequences using the NCBI Blast E-mail server. The *BlastP* program was used for protein sequence and *BlastN* was used for DNA sequence (27). All sequence comparisons and analyses were done using the Genetics Computer Group (GCG) software.

Northern and Southern blot analysis. 40 μg total RNA isolated from potato tubers (26) was run on a 1% denaturing agarose gel and transferred to GeneScreen Nylon Membranes (NEN) using capillary action as described by Maniatis *et al.* (25). Prehybridization and hybridization were performed in 50% formamide and washing was performed in SSC and SDS as described by Maniatis *et al.* (25).

Genomic DNA from potato leaves (var. Red dale) and *Arabidopsis* leaves (generously supplied by Dr. Robert Pruitt, Harvard University, Cambridge, MA) was isolated using the GREEN-GENE Plant DNA Isolation Kit (Clontech). 25 μg of genomic DNA from potatoes was digested with 150 Units of EcoRI and HindIII in separate reactions, and 25 μg of genomic DNA from *Arabidopsis* was digested with 150 Units of EcoRI. Prehybridization and hybridization were carried out as described above. For the *Arabidopsis* Southern, 30% formamide was used.

RESULTS

Purification of apyrase. The potato tuber apyrase was isolated as described by Kettlun *et al.* (18) with an additional sucrose gradient centrifugation step (Table 1). Starting with 1 kg of peeled red skin potatoes, a four step protocol was used to enrich the specific activity of the apyrase 10,000-fold from ~1 μmol P_i/mg/min in the crude extract to ~10,000 μmol P_i/mg/min. At this stage of the purification, SDS-PAGE showed a main band at ~50 kDa and a minor band at ~40 kDa (Fig. 1). The molecular mass of the native and denatured protein has been determined to be ~45–50 kDa

TABLE I
Potato Apyrase Purification Protocol

Purification step	Protein (mg)	Total activity (μmol/min)	Specific activity (μmol/mg/min)	Purification (–fold)	Yield (%)
Crude extract	13,000	13,500	1	NA	100
$(NH_4)_2SO_4$ fractionation	25	2,000	80	80	15
Gel filtration column	0.52	1,000	2,000	25	7.5
Cibracon blue column	0.059	350	6,000	3	2.5
Sucrose gradient	0.0015	15	10,000	1.7	0.1

using irradiation inactivation rates (28), gel filtration (18), sedimentation velocities (29), and SDS polyacrylamide gel electrophoresis (19). Assuming a molecular mass of 50 kDa and using the reported K_M for ATP of 10^{-4} M in the presence of Ca^{2+} (18), we estimate a turnover number of $\sim 10^4$ s^{-1} and a $k_{cat}K_M$ of $\sim 10^8$ $M^{-1}s^{-1}$.

The 50 kDa band was digested with trypsin and five peptides were sequenced. No amino terminal sequence could be obtained. Nucleotide sequences corresponding to the two longest peptides (Fig. 2, peptides #1 and #2) were amplified by PCR. Subsequent attempts to amplify the region between these two DNA sequences resulted in a 131 bp product and the discovery that the peptides are adjacent to each other. Tryptic digestion of the 40 kDa band gave an almost identical HPLC peptide map to that of the 50 kDa band (data not shown) indicating that both represent the same protein with the 40 kDa band most likely a degradation product.

cDNA library screening. The 131 bp cDNA sequence corresponding to most of the region coding for peptides #1 and #2 was used to probe a potato tuber cDNA library generously given to us by Dr. Normand Brisson (University of Montreal, Quebec). A single clone consisting of 1530 bp was isolated. The first ATG occurs 45 bp in from the 5′ end and is assumed to be the translation initiation site based on two criteria: the upstream sequence is not too divergent from the Kozak initiation site consensus sequence (30), and the observed molecular mass of ~50 kDa and the

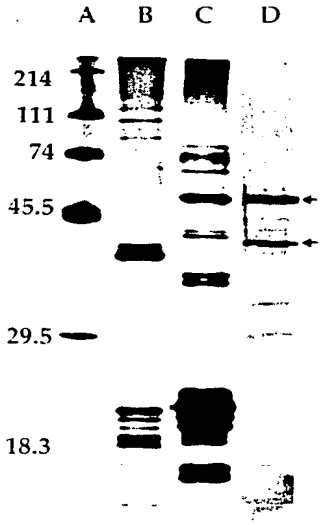

FIG. 1. SDS-PAGE of the potato apyrase purification fractions. Various steps of the purification protocol were analyzed on a 12.5% Laemmli gel (43). The protein bands were silver stained as described by Ansorge (44). (A) Prestained molecular weight markers with the apparent molecular masses indicated on the left in kilodaltons. (B) Approximately 5 μg of total protein from the crude extract. (C) Approximately 5 μg of total protein after the third ammonium sulfate fractionation step. (D) Approximately 10% of the sucrose density gradient fraction exhibiting the highest specific activity. The main 50 kDa and minor 40 kDa bands are indicated by arrows.

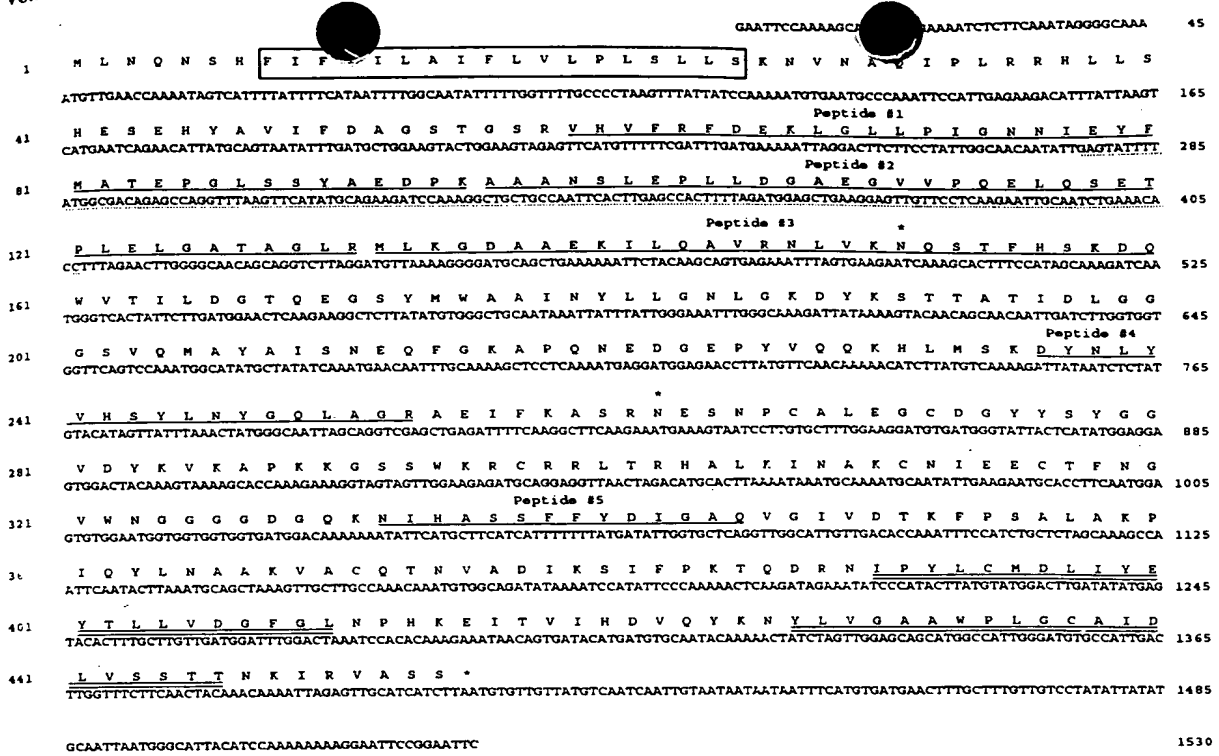

FIG. 2. Nucleotide and deduced amino acid sequence of the potato apyrase cDNA, RROP1. The peptides isolated by microsequencing are single underlined. The boxed amino acids represent the putative signal sequence with the predicted cleavage site indicated by the arrow (32). The two regions of hydrophobicity at the carboxy terminus are double underlined. Asterisks locate the potential Asn-linked glycosylation sites. The numbers on the left represent amino acids and the numbers on the right represent nucleotides. The nucleotide sequence single dot underlined (277–407) represents the region used as the probe in the screening of the cDNA library and all Northern and Southern blot analyses.

calculated molecular mass of 49.9 kDa from the deduced polypeptide sequence of 454 amino acids are in good agreement (Fig. 2). The deduced amino acid sequence contains all five of the peptides sequenced (Fig. 2). There is no consensus polyadenylation signal.

Hydropathy analysis using the Kyte-Doolittle algorithm (31) predicts three hydrophobic stretches in the protein. The first one at the amino terminus (residues (8–25) appears to represent a signal peptide with a predicted cleavage site after Ala-30 (32). The other two in the carboxy terminus are stretches of 21 amino acids (residues 390–410) and 20 amino acids (residues 427–446). Both are sufficiently long to traverse a membrane but whether they represent actual transmembrane domains has not been determined. There are two potential Asn-linked glycosylation sites at Asn-151 and Asn-262. Treatment of the purified apyrase with either N-glycanase or neuraminidase had no effect on the mobility of the protein in an SDS polyacrylamide gel indicating the protein is not heavily glycosylated (data not shown).

Sequence alignment. Using the NCBI *BlastP* program (27), seven similar protein sequences were identified: the two isoforms of an NTPase, NTP1 and NTP3, from a protozoan parasite (*Toxoplasma gondii*) (5), a nucleoside triphosphatase from garden pea (*Pisum sativum*) (33), a yeast golgi guanosine diphosphatase (*Saccharomyces cerevisiae*) (34), human CD39 (35), a hypothetical 71.9 kDa yeast protein (36), and a hypothetical 61.3 kDa protein from *Caenorhabditis elegans* (37). The potato apyrase showed 49, 33, 25, 23, 23, and 22% amino acid identity and 67, 52, 48, 52, 50, and 48% similarity with the garden pea NTPase, yeast GDPase, human CD39, yeast hypothetical protein, both *Toxoplasma* NTPases, and the *C. elegans* hypothetical protein, respectively. Analysis of all the sequences retrieved revealed four well conserved regions which we term ACR (apyrase

conserved regions (Fig. 3). None of the putative conserved sites show any striking similarity to the consensus Walker ATP binding motifs (38–40), but conserved region #1 and #4 are similar to the actin-hsp 70-hexokinase β-and γ-phosphate binding motifs, respectively (5,41).

The NCBI *BlastN* program (27) identified two sequences similar to the potato apyrase at the nucleic acid level: the garden pea NTPase (33) and a partial rice cDNA sequence (42).

Northern and Southern blot analysis. Total RNA isolated from potato tubers was probed with the 131 bp cDNA fragment (nucleotides 277–407). One band at ~1500–1600 bp was seen indicating a single RNA transcript of similar size to the cDNA clone (Fig. 4A). 25 μg of genomic DNA isolated from potato leaves was digested with EcoRI and HindIII. Southern blots of the digests showed multiple bands (Fig. 4B) indicating the potato apyrase is multigenic based on the observations that the restriction enzymes do not cut within the coding region and the 131 bp cDNA used as probe does not contain introns within the fragment at the level of the genomic DNA (data not shown). Genomic DNA from *Arabidopsis* was also examined under lower stringency hybridization and wash conditions; two bands were seen using the same probe as for the potato Southern analysis (Fig. 4C).

DISCUSSION

This manuscript describes the purification of a potato apyrase and the cloning of a cDNA encoding the potato enzyme. The identification of the cDNA as the apyrase gene is based on several criteria. First, the five peptides isolated from the purified apyrase are all present in the deduced polypeptide sequence. Second, the polypeptide is similar in sequence to known apyrases and related enzymes. Third, each of the four putative apyrase conserved regions share nearly identical sequence and spatial conservation with the related proteins (Fig. 3).

Although no functional studies on the ACR regions are available, the most reasonable hypothesis is that the conserved sites are involved in the catalytic activity of the apyrase. First, all of the similar proteins with known activities hydrolyze phosphoanhydride bonds of nucleoside tri- and di- phosphates (apyrases from potato, garden pea, and *T. gondii*) or nucleoside diphosphates (yeast GDPase). Second, ACR1 and ACR4 are similar to the actin-hsp 70-hexokinase β- and γ-phosphate binding motifs (5,41), indicating a possible role in nucleotide binding.

The mosquito apyrase which was recently cloned shows little similarity to the potato apyrase at the amino acid level, including the regions corresponding to the ACR sequences (Fig. 3). Champagne *et al.* (7) indicated that the deduced amino acid sequence of the salivary apyrase cDNA was similar to that of the 5′-nucleotidase protein family instead. Although the purified mosquito apyrase exhibits catalytic activity comparable to that of the other apyrases that have been cloned, it appears that this insect enzyme either evolved from a different ancestral progenitor than that for the proteins containing the ACR sites or that the cloned mosquito cDNA is a 5′-nucleotidase rather than an apyrase.

Interestingly, a yeast hypothetical 71.9 kDa protein sequenced from chromosome V, a *C. elegans* hypothetical 61.3 kDa protein sequenced from chromosome III, and a human lymphoid cell activation antigen, CD39, contain all four ACR regions and show high similarity with the apyrases at the amino acid level (Fig. 3). CD39 is primarily expressed on activated lymphoid cells and has been shown to mediate homotypic adhesion of B cells in the presence of anti-CD39 monoclonal antibodies (36). Currently, no enzymatic activity has been attributed to CD39, yet the possibility that this protein may also be responsible for the ecto-ATPase activity in human lymphoid cells (1) is intriguing.

Potato apyrase exists in both soluble and insoluble states that differ in their catalytic properties, isoelectric points, and slightly in molecular weights (15–19). We were only able to isolate a single cDNA from a potato tuber library, but Southern analysis on potato genomic DNA indicated the existence of multiple genes (Fig. 4B). Either there is only one active gene that can produce both isoforms or we have not isolated all the apyrase cDNA sequences.

A

Potato Apyrase	44	E	H	Y	A	V	I	F	D	A	G	S	T	G	S	R	V	H	V	F	R	F	D	65
Garden Pea NTPase	42	S	S	Y	A	V	V	F	D	A	G	S	T	G	S	R	I	H	V	Y	H	F	N	63
S. cerevisiae GDPase	91	H	K	Y	V	I	M	I	D	A	G	S	T	G	S	R	V	H	I	Y	K	F	D	112
T. gondii NTP1	63	L	Q	A	L	V	V	I	D	A	G	S	S	S	T	R	T	N	V	F	.	L	A	83
T. gondii NTP3	63	L	Q	A	L	V	V	I	D	A	G	S	S	S	T	R	T	N	V	F	.	L	A	83
Yeast 71.9 kDa	8	D	R	F	G	I	V	I	D	A	G	S	S	G	S	R	I	H	V	F	K	W	Q	29
C. elegans 61.3 kDa	42	R	S	Y	G	V	I	C	D	A	G	S	T	G	T	R	L	F	V	Y	N	W	I	63
Human CD39	47	V	K	Y	G	I	V	L	D	A	G	S	S	H	T	S	L	Y	I	Y	K	W	P	68
Murine CD39	47	V	K	Y	G	I	V	L	D	A	G	S	S	H	T	N	L	Y	I	Y	K	W	P	68
Mosquito Apyrase	32	K	D	V	S	K	L	F	P	L	T	L	I	H	I	N	D	L	H	A	R	F	E	53

B

Potato Apyrase	120	.	T	P	L	E	L	G	A	T	A	G	L	R	M	L	K	G	D	.	.			136
Garden Pea NTPase	118	.	T	P	V	R	L	G	A	T	A	G	L	R	L	L	N	G	D	.	.			134
S. cerevisiae GDPase	165	.	T	P	V	A	V	K	A	T	A	G	L	R	L	L	G	D	A	.	.			181
T. gondii NTP1	180	G	I	P	V	M	L	C	S	T	A	G	V	R	D	F	H	E	.	.	.			196
T. gondii NTP3	180	G	I	P	V	M	L	C	S	T	A	G	V	R	D	F	H	E	.	.	.			196
Yeast 71.9 kDa	100	.	C	P	V	F	I	Q	A	T	A	G	M	R	L	L	P	Q	D	I	.			117
C. elegans 61.3 kDa	124	.	T	P	V	F	I	F	A	T	A	G	M	R	L	I	P	D	E	Y	V			142
Human CD39	124	.	T	P	V	Y	L	G	A	T	A	G	M	R	L	L	R	M	E	S	E			142
Murine CD39	124	.	T	P	V	Y	L	G	A	T	A	G	M	R	L	L	R	M	E	S	E			142
Mosquito Apyrase	150	E	G	I	P	T	I	V	A	N	L	V	M	N	N	D	P	D	L	K	S			169

C

Potato Apyrase	165	.	L	D	G	T	Q	E	G	S	Y	M	W	A	A	I	N	Y	L	L	G	N	L	G	K	186
Garden Pea NTPase	163	.	I	D	G	T	Q	E	G	S	Y	L	W	V	T	V	N	Y	A	L	G	N	L	G	K	184
S. cerevisiae GDPase	211	.	M	G	G	D	E	E	G	V	F	A	W	I	T	T	N	Y	L	L	G	N	I	G	A	232
T. gondii NTP1	230	P	I	T	G	A	E	E	G	L	F	A	F	I	T	L	N	H	L	S	R	R	L	G	E	253
T. gondii NTP3	230	P	I	T	G	A	E	E	G	L	F	A	F	I	T	L	N	H	L	S	R	R	L	G	E	253
Yeast 71.9 kDa	147	.	I	D	G	E	T	E	G	L	Y	G	W	L	G	L	N	Y	L	Y	G	H	F	N	D	169
C. elegans 61.3 kDa	174	.	I	E	G	K	W	E	G	I	Y	S	W	I	A	V	N	Y	A	L	G	K	F	N	K	196
Human CD39	169	.	I	T	G	Q	E	E	G	A	Y	G	W	I	T	I	N	Y	L	L	G	K	F	S	Q	183
Murine CD39	169	.	I	T	G	Q	E	E	G	A	Y	G	W	I	T	I	N	Y	L	L	G	R	F	T	Q	183
Mosquito Apyrase	205	T	L	S	N	A	V	E	A	V	.	R	R	E	A	A	A	L	K	K	D	K	I	D	I	227

D

Potato Apyrase	192	.	T	T	A	T	I	D	.	.	L	G	G	G	S	V	Q	M	A	Y	A	I	S	N	E	212
Garden Pea NTPase	190	.	T	V	G	V	I	D	.	.	L	G	G	G	S	V	Q	M	A	Y	A	V	S	K	K	210
S. cerevisiae GDPase	239	P	T	A	A	V	F	D	.	.	L	G	G	G	S	T	Q	I	V	F	.	.	.	E	P	157
T. gondii NTP1	271	D	L	A	G	V	V	E	.	.	V	G	G	A	S	A	Q	I	V	F	P	L	Q	E	G	192
T. gondii NTP3	271	D	L	A	G	V	V	E	.	.	V	G	G	A	S	A	Q	I	V	F	P	L	Q	E	G	192
Yeast 71.9 kDa	178	F	T	F	G	F	M	D	.	.	M	G	G	A	S	T	Q	I	A	F	A	P	H	D	S	199
C. elegans 61.3 kDa	213	K	T	V	G	M	I	D	.	.	M	G	G	A	S	A	Q	I	A	F	E	L	P	D	T	234
Human CD39	207	E	T	F	G	A	L	D	.	.	L	G	G	A	S	T	Q	V	T	F	V	P	Q	N	Q	228
Murine CD39	207	E	T	F	G	A	L	D	.	.	L	G	G	A	S	T	Q	I	T	F	V	P	Q	N	S	228
Mosquito Apyrase	245	E	A	G	D	D	I	D	V	I	V	G	A	H	S	H	S	F	L	Y	S	P	D	S	K	268

FIG. 3. Sequence analysis of the putative apyrase conserved regions (ACRs) of the potato apyrase and related proteins. The amino acid sequences from potato apyrase, garden pea nucleoside triphosphatase, *Saccharomyces cerevisiae* golgi guanosine diphosphatase, *Toxoplasma gondii* nucleoside triphosphate hydrolase isoforms (NTP1 and NTP3), a yeast hypothetical 71.9 kDa protein, a *Caenorhabditis elegans* hypothetical 61.3 kDa protein, human CD39, murine CD39 (35), and mosquito salivary apyrase were aligned using the GCG *Pileup* program. The boxed areas represent the core regions of high homology. (A) Putative apyrase conserved region #1 (ACR1). (B) ACR2. (C) ACR3. (D) ACR4.

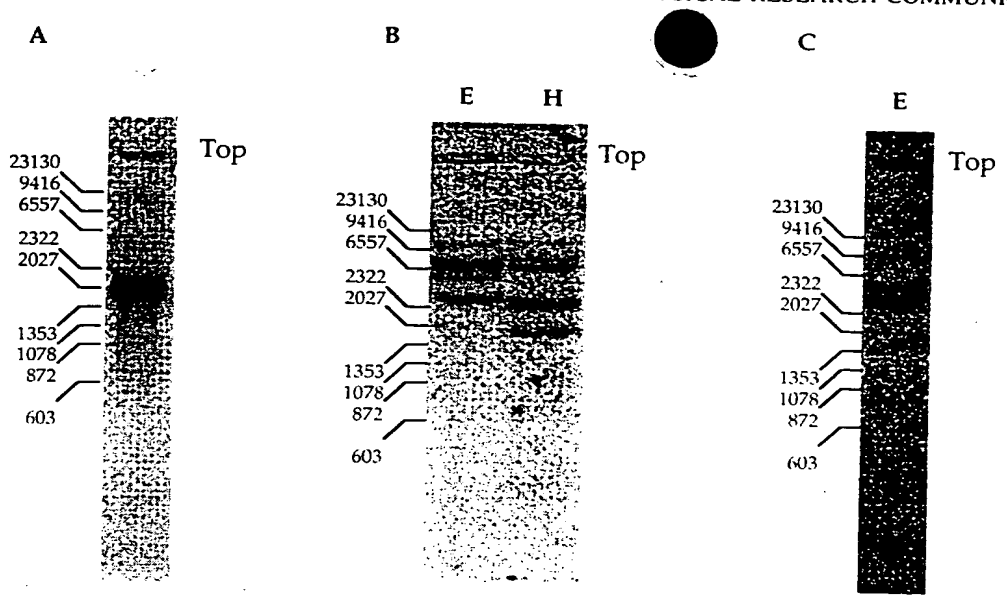

FIG. 4. Northern and Southern blot analysis in potatoes and *Arabidopsis*. All blots were probed with the 131 bp PCR product, nucleotides (277–407). The top of each gel is labeled. The molecular markers are indicated on the left in base pairs. (A) Northern analysis of 40 μg total RNA from potato tubers. (B) Southern analysis of 25 μg potato genomic DNA digested with EcoRI(E) and HindIII(H). (C) Southern analysis of 25 μg *Arabidopsis* genomic DNA digested with EcoRI(E).

In *T. gondii*, the mature forms of the NTP1 and NTP3 gene products are soluble proteins that are able to associate with membranes. The isoforms are 97% identical at the amino acid level and are predicted to undergo cleavage of their putative signal sequences based on the reduced molecular mass of the *in vitro* translated NTP1 polypeptide in the presence of microsomes (5). Immunofluorescence studies showed that both NTPase were located within dense granules of the protozoan parasite and upon host cell infection were secreted into the parasitophorous vacuole where a fraction of both enzymes remained soluble and a fraction associated with the parasitophorous vacuolar membrane (PVM) (5). It has been shown that one of the membrane associated apyrases from potato can be fully solubilized in 2 M NaCl indicating a peripheral attachment to the membrane as opposed to an integral one (19).

We are currently attempting to express an active potato apyrase in COS-7 cells and are planning to isolate related genes in animal cells by cloning through homology. The possibility of an apyrase homolog in *Arabidopsis* and *C. elegans* now opens up avenues for genetic manipulations to study the physiology of the apyrases.

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